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liposome same load\$ same (platinu or cisplatin)	64

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<u>L3</u>	liposome same load\$ same (platinu or cisplatin)	64	<u>L3</u>
<u>L2</u>	liposome same temperature same (platinu or cisplatin)	27	<u>L2</u>
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L1 and cycle	53

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result set*DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI,TDBD; PLUR=YES; OP=OR*L2

L1 and cycle

53

L2L1

liposome same cooling same heating

93

L1

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L1: Entry 25 of 93

File: PGPB

Aug 7, 2003

DOCUMENT-IDENTIFIER: US 20030147945 A1

TITLE: Compositions for delivery of drug combinations

Detail Description Paragraph:

[0238] Cisplatin was passively entrapped in liposomes by first solubilizing the drug (at 40 mg/mL) in a solution consisting of 150 mM CuCl.sub.2, 20 mM histidine (pH 7.4, pH adjusted with triethanolamine) plus 4% (v/v) DMSO and heating the resulting solution to 80.degree. C. to enhance the solubility of cisplatin. The cisplatin solution was then added at 80.degree. C. to a lipid film composed of DMPC and cholesterol with trace levels of .sup.14C-CHE. The hydrated lipid films were extruded at 80.degree. C. through two 100 nm filters and the liposomes cooled to room temperature. Upon cooling, the samples were centrifuged in a bench top centrifuge at 2000.times.g for 5 minutes to pellet any unencapsulated cisplatin, and the supernatant collected. Removal of excess metal ions was carried out by passage through a Sephadex G-50 gel filtration column and collection of the liposome fraction.

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L1: Entry 30 of 93

File: PGPB

Feb 20, 2003

DOCUMENT-IDENTIFIER: US 20030035842 A1

TITLE: Lipobeads and their production

Brief Description of Drawings Paragraph:

[0024] FIG. 10 shows graphs of (a) the Z-average diameter of liposomes as a function of temperature during heating/cooling cycles and (b) pH Dependence of the average diameter of the liposomes.

Detail Description Paragraph:

[0055] The temperature dependence of the apparent diameter of egg phosphatidylcholine (EPC) liposomes between 20 and 40.degree. C. is shown in FIG. 10a. In the temperature range above the main transition temperature (-2.degree. C.), there was a slight decrease in <d> with an increasing temperature. The decrease was less than 5% in the range studied. There was almost no difference in <d> between the heating (solid circles) and the cooling (open circles), indicating a reversible change without thermal hysteresis.

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File: PGPB

Dec 5, 2002

DOCUMENT-IDENTIFIER: US 20020182248 A1

TITLE: Liposomes and liposomal dispersion

Summary of Invention Paragraph:

[0043] The liposomes which have a sphingolipid as the main component of the liposomal membrane-constituting lipids and contain a drug of interest, and a dispersion-of the liposomes, may be produced by dissolving the drug in an organic solvent together with the lipid component, making the mixture dried, and then mixing it with the aqueous solvent to make a dispersion of liposomes, or by adding an aqueous solution of the drug to a dried lipid film of the lipid component prepared in advance, allowing the mixture to stand for a predetermined period of time, preferably by heating it above the phase transition temperature (Tc) of the membrane, and then cooling it spontaneously.

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L1: Entry 53 of 93

File: USPT

Jun 5, 2001

DOCUMENT-IDENTIFIER: US 6241999 B1

**\*\* See image for Certificate of Correction \*\***

TITLE: Method for producing liposomes with increased percent of compound encapsulated

Detailed Description Text (75):

Multilamellar liposomes (MLV) were prepared by adding 20 mg/mL cytarabine (The Upjohn Co., Kalamazoo, Mich.) solution preheated to 60-65.degree. C. into a test tube containing one of a series of test phosphatidylcholine (PC) having chain lengths ranging from 14 to 18 carbons (DCn:OPC, n=14-18) to make a 100 mM lipid dispersion. At 10 minute intervals for a total of five times, the dispersion was stirred in the test tube for 30 seconds using a vortexer (Baxter S/P Vortex Mixer) at the maximum speed. The dispersion was then allowed to undergo three cooling-heating cycles across the phase transition temperature of the test PC to facilitate drug equilibration across the bilayer membranes of the liposomes. The MLVs were then pelleted by centrifugation at 600.times.g and washed with normal saline (20:1 volume ratio). To ensure appropriate pellet washing, saline wash tests were conducted at various washing temperatures. It was found that a relatively thorough wash can be achieved with only two saline washes if the washing temperature is kept below the gel-liquid crystalline transition temperature of the test PC. For this reason, MLV made using DC14:OPC as the test PC were washed at 4.degree. C., and those made using DC16:OPC or DC18:OPC as the test PC were washed at the ambient temperature. After the wash, the pellet was resuspended in normal saline.

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L1: Entry 55 of 93

File: USPT

Feb 27, 2001

DOCUMENT-IDENTIFIER: US 6193998 B1

**\*\* See image for Certificate of Correction \*\***

TITLE: Method for producing liposomes with increased percent of compound encapsulated

Detailed Description Text (76):

Multilamellar liposomes (MLV) were prepared by adding 20 mg/mL cytarabine (The Upjohn Co., Kalamazoo, Mich.) solution preheated to 60-65.degree. C. into a test tube containing one of a series of test phosphatidylcholine (PC) having chain lengths ranging from 14 to 18 carbons (DCn:0PC, n=14-18) to make a 100 mM lipid dispersion. At 10 minute intervals for a total of five times, the dispersion was stirred in the test tube for 30 seconds using a vortexer (Baxter S/P Vortex Mixer) at the maximum speed. The dispersion was then allowed to undergo three cooling-heating cycles across the phase transition temperature of the test PC to facilitate drug equilibration across the bilayer membranes of the liposomes. The MLVs were then pelleted by centrifugation at 600.times.g and washed with normal saline (20:1 volume ratio). To ensure appropriate pellet washing, saline wash tests were conducted at various washing temperatures. It was found that a relatively thorough wash can be achieved with only two saline washes if the washing temperature is kept below the gel-liquid crystalline transition temperature of the test PC. For this reason, MLV made using DC14:0PC as the test PC were washed at 4.degree. C., and those made using DC16:0PC or DC18:0PC as the test PC were washed at the ambient temperature. After the wash, the pellet was resuspended in normal saline.

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L2: Entry 45 of 53

File: USPT

Dec 7, 1999

DOCUMENT-IDENTIFIER: US 5997899 A

TITLE: Method for producing liposomes with increased percent of compound encapsulated

Detailed Description Text (75):

Multilamellar liposomes (MLV) were prepared by adding 20 mg/mL cytarabine (The Upjohn Co., Kalamazoo, Mich.) solution preheated to 60-65.degree. C. into a test tube containing one of a series of test phosphatidylcholine (PC) having chain lengths ranging from 14 to 18 carbons (DCn:OPC, n=14-18) to make a 100 mM lipid dispersion. At 10 minute intervals for a total of five times, the dispersion was stirred in the test tube for 30 seconds using a vortexer (Baxter S/P Vortex Mixer) at the maximum speed. The dispersion was then allowed to undergo three cooling-heating cycles across the phase transition temperature of the test PC to facilitate drug equilibration across the bilayer membranes of the liposomes. The MLVs were then pelleted by centrifugation at 600.times.g and washed with normal saline (20:1 volume ratio). To ensure appropriate pellet washing, saline wash tests were conducted at various washing temperatures. It was found that a relatively thorough wash can be achieved with only two saline washes if the washing temperature is kept below the gel-liquid crystalline transition temperature of the test PC. For this reason, MLV made using DC14:OPC as the test PC were washed at 4.degree. C., and those made using DC16:OPC or DC18:OPC as the test PC were washed at the ambient temperature. After the wash, the pellet was resuspended in normal saline.

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L2: Entry 17 of 27

File: USPT

Oct 3, 2000

DOCUMENT-IDENTIFIER: US 6126966 A

TITLE: Liposomes containing a cisplatin compound

Brief Summary Text (20):

Cisplatin, however, is difficult to efficiently entrap in liposomes because of the drug's low aqueous solubility, approximately 1.0 mg/ml at room temperature, and low lipophilicity, both of which contribute to a low drug/lipid ratio.

Brief Summary Text (28):

In another aspect, the invention includes a method of entrapping a cisplatin compound in liposomes, by heating an aqueous solution of a cisplatin compound to a temperature sufficient to increase its solubility over the compound's solubility at room temperature. To the heated cisplatin compound solution is added a vesicle-forming lipid and between about 1-20 mole percent of a vesicle-forming lipid derivatized with a hydrophilic polymer. By said adding, liposomes having an inner surface coating and an outer surface coating of hydrophilic polymer chains are formed and the cisplatin compound is entrapped in the liposomes with substantially greater retention than in liposomes lacking the polymer coatings.

Detailed Description Text (13):

Lipids suitable for use in the cisplatin liposome composition of the present invention include vesicle-forming lipids having phase transition temperatures at or below room temperature and those having a high phase transition temperature. In a preferred embodiment, a vesicle-forming lipid having a phase transition temperature between about 40-70.degree. C. is employed. In another embodiment, the lipid used in forming the liposomes is one having a phase transition temperature within about 20.degree. C., more preferably 10.degree. C., most preferably 5.degree. C., of the temperature to which the solution containing the cisplatin compound is heated during liposome preparation, as will be described. Phase transition temperatures of lipids are tabulated in a variety of sources, such as Avanti Polar Lipids catalogue and Lipid Thermotropic Phase Transition Database (LIPIDAT, NIST Standard Reference Database 34).

Detailed Description Text (18):

In the example detailed below, the vesicle-forming lipid HSPC, the derivatized vesicle-forming lipid PEG-DSPE and cholesterol are dissolved in ethanol heated to about 65.degree. C., just above HSPC phase transition temperature's between about 52-60.degree. C. An aqueous solution of native cisplatin is heated to between 63-67.degree. C. The solutions are mixed together to form liposomes containing the cisplatin compound in entrapped form. The method of the invention achieves a high encapsulation of cisplatin, typically encapsulating between 10-20 .mu.g drug/mg lipid, and provides liposomes having, in addition to the outer surface coating, an inner surface coating of hydrophilic polymer chains, with the cisplatin compound stably entrapped within the liposome.

Detailed Description Text (59):

The cisplatin solution and the lipid solution were added together to form liposomes, with the temperature of the mixture maintained at between 60-65.degree. C.

Detailed Description Text (60):

The liposomes, following diafiltration and dialysis, were extruded through 0.2 .mu.m and 0.1 .mu.m polycarbonate filters to size the liposomes to between about 100-120 nm. The liposome suspension was cooled to room temperature, and the untrapped, precipitated cisplatin was removed by filtering and by diafiltration.

Detailed Description Text (63):

The stability of liposomes prepared as described above (Example 3) was evaluated by (i) analyzing the liposomal suspension for cisplatin and platinum concentrations, (ii) determining percent of encapsulated platinum, (iii) measuring liposome size, and (iv) measuring the pH of the liposome suspension, each as a function of time and temperature.

Detailed Description Text (66):

Liposomes stored at temperatures of -40.degree. C. and -20.degree. C. showed no measurable loss in the concentrations of cisplatin or platinum after storage for three months. Nor was a significant change observed in liposome size or suspension pH after the three month storage period. At -20.degree. C. and after 18 months of storage, the cisplatin was retained in the liposomes, as evidenced by no significant loss in either the cisplatin and platinum concentration. The liposome composition was also stable when stored at 2-8.degree. C. for 18 months. As seen in Table 1, no measurable losses in the concentrations of cisplatin or platinum were observed. At the 18 month time point, the percentage of encapsulated platinum was 99%, indicating that all but 1% of the platinum was retained in the liposomes. That the entrapped platinum is in the form of cisplatin is evident from the cisplatin concentration, which does not decrease over the 18 month storage period.

Detailed Description Text (84):

From the foregoing, it can be appreciated how various features and objects of the invention are met. The liposome composition of the present invention includes liposomes having an inner surface coating and an outer surface coating of hydrophilic polymer chains and an entrapped cisplatin compound. The studies performed in support of the invention demonstrate that the cisplatin is stably entrapped in the liposomes, as evidenced by retention of cisplatin and platinum after incubation for various times at several temperatures. Cisplatin stably entrapped in liposomes offers the advantages of reduced toxicity and of improved efficacy, relative to the drug administered in free form, since after administration the drug remains entrapped in the liposome with little leakage to the bloodstream. The outer surface coating of PEG chains provide a long blood circulation lifetime allowing the liposomes to reach a target site, such as a tumor.

Detailed Description Text (134):

A suspension of cisplatin-containing liposomes was prepared as described in Example 3. 5 ml aliquots of the liposome suspension were placed in 10 cc glass vials, sealed under aseptic conditions, and placed in incubators or refrigerators at the following temperatures: -40.degree. C., -20.degree. C., 2-8.degree. C., 30.degree. C., 40.degree. C. At intervals, samples from each vial stored at each temperature were drawn and tested in triplicate for:

Detailed Description Paragraph Table (1):

TABLE 1 <u>Stability of Cisplatin-Containing Liposomes</u>														
<u>Cisplatin</u>	<u>Pt</u>	<u>%</u>	<u>Liposome</u>	<u>Time</u>	<u>Conc.</u>	<u>Conc.</u>	<u>Encapsulated</u>	<u>size</u>	<u>Temp.</u>	<u>(month)</u>				
<u>(mg/ml)</u>	<u>(mg/ml)</u>	<u>Platinum</u>	<u>(nm)</u>	<u>pH</u>										
-40.degree.														
C. 0	0.92	0.71	100	109	6.50	1	0.86	0.71	99	106	6.55	3	0.86	-- -- 114 6.49 -
20.degree. C.	1	0.85	0.71	100	111	6.56	3	0.86	-- --	117	6.49	18	1.00	0.69 -- 117
6.29	2-8.degree. C.	1	0.85	0.71	100	109	6.61	3	0.87	0.68	100	109	6.48	6 0.90 0.73
99	110	6.54	18	0.86	0.71	99	109	6.30	30.degree. C.	1	0.70	0.71	98	107 6.43 3 0.55
0.68	93	107	6.18	40.degree. C.	0.5	0.65	0.71	96	110	6.36	1	0.53	0.65	91 106 6.25

Detailed Description Paragraph Table (2):

TABLE 2 Stability of PEG-Coated Liposomes  
and Comparative Liposomes at 60.degree. C. for 6 Hours Incubation Cisplatin %  
Temperature Conc. Encapsulated Size Formulation and Time (mg/ml) Platinum (nm) pH  
HSPC/chol/ 0 0.38 100 116 -- mPEG-DSPE  
60.degree. C.; 0.29 96 117 -- 6 hours HSPC/chol/ 0 0.25 100 149 6.53 DSPG  
60.degree. C.; 0.14 82 148 6.62 (comparative 6 hours composition)

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Detailed Description Paragraph Table (3):

TABLE 3 Stability of PEG-Coated Liposomes  
and Comparative Liposomes at 40.degree. C. for 2 Weeks Incubation Cisplatin %  
Temperature Conc. Encapsulated Size Formulation and Time (mg/ml) Platinum (nm) pH  
HSPC/chol/ 0 0.75 100 108 6.62 mPEG-DSPE  
40.degree. C.; 0.53 95 114 6.09 2 weeks HSPC/chol/ 0 0.51 100 146 6.53 DSPG  
40.degree. C.; 0 81 137 5.84 (comparative 2 weeks composition)

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L2: Entry 13 of 27

File: PGPB

Oct 25, 2001

DOCUMENT-IDENTIFIER: US 20010033861 A1

TITLE: Liposomes containing an entrapped compound in supersaturated solution

Detail Description Paragraph:

[0038] Accordingly, compounds contemplated for use in the compositions and methods of the invention include compounds capable of at least about a two-fold, preferably at least about three-fold, more preferably at least about a four-fold increase in room temperature (20-25.degree. C.) aqueous solubility via: (i) increasing solvent temperature, (ii) addition of a co-solvent, or by (iii) changing solvent pH. Preferred compounds are those with limited solubility in water at room temperature that undergo a substantial increase in solubility with increase in temperature. In the studies performed in support of the invention, cisplatin was used as the model compound. Cisplatin has a solubility at room temperature in water or saline of 1-2 mg/mL. At about 60-65.degree. C., the solubility increases to about 8-10 mg/mL. Thus, temperature was used as the means to prepare a concentrated solution of the drug for preparation of liposomes.

Detail Description Paragraph:

[0046] As described above, the invention provides efficient and stable encapsulation of drugs having limited water solubility at room temperature. Such compounds are known to be difficult to entrap in liposomes in a drug-to-lipid ratio useful for therapy. In the studies performed in support of the invention, the anti-cancer agent cisplatin was used as an exemplary drug. It should be appreciated, however, that the composition and method of the invention are contemplated for use with a variety of compounds, which have limited water solubility at room temperature, and other exemplary drugs are described above.

Detail Description Paragraph:

[0047] Cisplatin (cis-diamminedichloroplatinum(II)), is a heavy metal complex containing a central atom of platinum surrounded by two chloride atoms and two ammonia molecules in the cis position. Cisplatin is widely used for treating a variety of solid tumors, including testicular, head and neck, and lung tumors. Like other cancer chemotherapeutic agents, cisplatin is a highly toxic drug. The main disadvantages of cisplatin are its extreme nephrotoxicity, which is the main dose-limiting factor, its rapid excretion via the kidneys, with a circulation half life of only a few minutes, and its strong affinity to plasma proteins (Freise, J., et al., Arch. Int. Pharmacodyn., 258:180-192 (1982)). Encapsulation of cisplatin in liposomes as an approach to overcoming toxicity has been described for example in Abra, et al., U.S. Pat. No. 5,945,122; Gondal, J. A., et al., Eur. J. Cancer, 29A (11):1536-1542 (1993); Sur, B., et al., Oncology, 40:372-376 (1983); Weiss, R. B., et al., Drugs, 46(3):360-377 (1993). Cisplatin is typically difficult however, to efficiently entrap in liposomes because of the drug's low aqueous solubility, approximately 1-2 mg/mL at room temperature, and low lipophilicity, both of which contribute to a low drug/lipid ratio.

Detail Description Paragraph:

[0048] In accordance with the invention, liposome-containing cisplatin in a supersaturated state was prepared as described in Example 1. Briefly, an aqueous solution of the drug was prepared with the drug at a concentration above its solubility limit in the solution at room temperature, about 20-25.degree. C., at

pH=7. In this case, the solubility of the drug was increased by heating the temperature of the solution. Other methods to increase the solubility of a drug in an aqueous solution at room temperature, as discussed above, are also suitable. The prepared solution is subsequently used to hydrate lipids selected for formation of the liposome lipid bilayer, thereby forming liposomes containing the supersaturated drug solution in the central core compartment of the liposomes. More specifically, and in the particular case using cisplatin, the liposomes contained cisplatin entrapped at a concentration of about 8 mg/ml, about four times above cisplatin solubility at room temperature (1-2 mg/mL). The liposomes were sized by extrusion to an average mean particle diameter of 106 nm.

Detail Description Paragraph:

[0052] Liposomes containing .sup.15N-labeled cisplatin were prepared as described in Example 2. The drug loading was performed at a temperature of between 60-65.degree. C. to increase the cisplatin solubility to about 8 mg/mL. After formation of large unilamellar vesicles, the temperature of the liposome suspension was lowered to 4.degree. C. at which the drug solubility is about 1-2 mg/mL.

Detail Description Paragraph:

[0058] .sup.31P NMR was used to verify the state of phospholipids in the lipid vesicles, as well as to verify the homogeneity of liposomal preparations with respect to size distribution and the number of lamellae. The .sup.31P NMR spectra of the liposome formulations for which samples were also measured by .sup.195Pt NMR are described in Table 1 were measured; they displayed the slightly asymmetric peaks typical of phospholipid vesicles. The same samples were measured by .sup.195Pt NMR to verify their platinum content. At room temperature, the .sup.31P peak observed was very broad. This is in agreement with previous data of vesicles composed of saturated phospholipids and cholesterol below the gel-to-liquid crystalline phase transition temperature.<sup>TM</sup> of the matrix lipids ( $T_m=52.5$ .degree. C. for hydrogenated phosphatidylcholine) (Lichtenberg D. et al., Methods of Biochemical Analysis, D. Glick, Ed., Vol. 23, Wiley, New York, p. 337 (1998)). As seen in FIGS. 3A-3B, at 60.degree. C., above the  $T_m$ , sharp .sup.31P spectra were observed for both the platinum containing (FIG. 3A) and control (FIG. 3B) liposomes. At 60.degree. C., approximately 10 scans were sufficient to produce reliable data with a high signal-to-noise ratio. Peak analysis revealed somewhat asymmetric peaks for both (skewness to the right), as is expected for phospholipid vesicles. Measurement of the linewidths at half the height can serve as a criterion for the interaction of the platinum with the phospholipids in the formulation. Linewidths at half the height ( $\Delta\nu_{1/2}$ ) were determined at 60.degree. C., and values of 6.3 ppm and 4.2 ppm were obtained for the control and for cisplatin-liposomes, respectively. Comparison between the Pt-containing liposomes and the control liposomes indicated minor interactions between the platinum and the phospholipids. A weak interaction may occur, as  $\Delta\nu_{1/2}$  Pt-liposomes <  $\Delta\nu_{1/2}$  control, although both vesicles are of similar size. The data are in good agreement with .sup.31P measurements of 100-200 nm diameter LUV (i.e.  $\Delta\nu_{1/2}$  about 5 ppm for egg PC/DSPG 85/15  $\Delta\nu_{1/2}$ ) [Tilcock, C. P. S., Chem. Phys. Lipids 40:109 (1986)]. The linewidth suggests no major contamination with MLV. Thus, .sup.31P NMR has shown by the shape of the peaks that there are no phospholipids in a hexagonal type II state.

Detail Description Paragraph:

[0071] The liposomes for use in this study were prepared in the presence of ethanol during the first stages of liposome formulation and a study was performed to determine the effect of ethanol on the solubility of cisplatin. Hence, the solubility of cisplatin at 1 and 8 mg/ml at room temperature and at 65.degree. C. in 0.9% NaCl and in 20% ethanol in 0.9% NaCl was examined. At 1 mg/ml, cisplatin was soluble under all conditions, while at 8 mg/ml, most of the cisplatin precipitated at room temperature, yet was mostly soluble at 65.degree. C. Lowering the temperature back to room temperature led to the precipitation of most of the 8 mg/ml of the cisplatin, in both the absence and presence of 20% ethanol. Thus, it

can be concluded that the presence of 20% ethanol did not improve the solubility of cisplatin. NMR measurements indicate that the solubility of free cisplatin in the aqueous phase is limited to .about.2 mg/ml, and is increased upon a rise in temperature to 60.degree. C. The NMR experiments show detection of a peak whose integration is proportional to .about.2 mg/ml, whereas the insoluble platinum precipitate is in fact undetected. In the case of the liposomes, nearly all the cisplatin accounted for by atomic absorption is soluble in the intraliposomal aqueous phase, which suggests that the intraliposomal concentration is higher than 2 mg/ml, which is the solubility at room temperature. It was found that in spite of the fact that the concentration of cisplatin during liposome preparation was above the solubility at room temperature (or 4.degree. C.), nearly all the cisplatin in the liposomes behaved as if soluble in the intraliposomal aqueous phase. From the solubility studies it is clear that ethanol is not responsible for the higher than expected drug-to-lipid ratio.

Detail Description Paragraph:

[0115] 8.5 mg/ml .sup.15N-labeled cisplatin was dissolved in 0.9% NaCl at 65.degree. C. and left at this temperature for 1 hour. Lipids (HSPC/cholesterol/.sup.2000PEG-DSPE 51:44:5) were dissolved in ethanol. The lipids were hydrated by adding this ethanolic solution to the drug mixture. Final lipid concentration was 150 mg/ml (15%) in 10% ethanol, at 65.degree. C. The mixture was kept stirring for 1 hour at 60.degree. C., then extruded at 65.degree. C. through 100 nm and 200 nm pore size polycarbonate filters using the Lipofast syringe extruder. Sized liposomes (.about.100 nm) were allowed to cool to room temperature. During the cooling, a heavy yellow precipitate formed. The supernatant was collected and allowed more standing-time at room temperature. More precipitation occurred, and the supernatant was collected again. The sample was diluted twofold and dialyzed 5 times against 100 volumes of 10% sucrose containing 1 mM NaCl at room temperature. Under these conditions, a complete equilibration with 10% sucrose containing 1 mM NaCl should occur. Finally, histidine buffer (pH 6.5) was added to a final concentration of 10 mM. The final liposome dispersion was translucent, white. Aliquots of the liposomes and of both precipitates were analyzed by heteronuclear single quantum coherence (HSQC) at 30.degree. C.

Detail Description Paragraph:

[0122] .sup.31P measurements were carried out at room temperature and at 60.degree. C. using a 5 mm computer-switchable probe. The .sup.31P chemical shifts were measured relative to phosphoric acid set at 0 ppm. Data were collected with broadband decoupling of the protons, using 10000 Hz spectral width, and an acquisition time of 1.6 s. Usually, 250 pulses or more were acquired and a line broadening of 20.0 Hz was applied. Placebo (control) and cisplatin-loaded liposomes were studied by .sup.31P NMR. The liposomes contained large unilamellar vesicles (LUV) and were composed of hydrogenated phosphatidylcholine (HPC), cholesterol, and .sup.2000PEG-DSPE at a mole ratio of 55:40:5. Samples were prepared by mixing 700 .mu.l of the original liposome sample with 70 .mu.l of D.sub.2O.

Detail Description Paragraph:

[0136] Freeze-dried cisplatin liposomes were prepared as follows. A sample of cisplatin-containing liposomes was dialysed using molecular weight cut-off 14,000 dialysis tubing and four, twenty-volume exchanges of 10% (w/v) sucrose solution over twelve hours at room temperature. The resulting liposome suspension in 10% sucrose solution was then rapidly frozen using a dry-ice/isopropanol mixture and lyophilized overnight at high vacuum (100 mTorr).

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